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The Partial Enzymatic Hydrolyses of Yeast Nucleic Acid.

Dissertation

Submitted to the Board of University Studies of the Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy.

By

Annabella E. Richards.

1914

169,104

Acknowledgment

The author wishes to express her appreciation of the advice and assistance rendered by Professor [illegible] during the course of this investigation.

In the year 1874 the distinguished hygiologist Friedrich Miescher[1] made an extensive study of the habits of the Rhine salmon in fresh water. By comparative observations of animals captured at various points of the river between its mouth and the spawning beds at Frankfurt, he was able to prove an older suspicion that these fish never feed while ascending the river; for, with very rare and easily explained exceptions the alimentary canal was found free from food detritus [illegible] had greatly decreased during this journey, while both the male and female organs of reproduction grown enormously. Miescher drew the obvious at the expense of muscle protein; so that the principle

① F. Miescher (1874). Die Spermatozoen einiger Wirbelthiere. Verhandl. d. naturforsch. Ges in Basel, 6, 138. Die histochemischen und

scribed to a protein substance.

During the spawning season the spermatic fluid (lachamilch) can be obtained from these animals in [illegible] "Vas deferens" of a living fish consists of little more than spermatozoa suspended in a dilute salt solution. Upon [illegible] consist of head, tail, and connecting part "Mittelstück", it being especially characteristic that the two latter parts [illegible] comparison with the mass of the head, while the tails are threads of extreme fineness which may be easily dissolved with acetic acid, leaving the heads as [illegible] granular masses.

From comparative histological studies of the growing testicle it becomes apparent that the spermatozoa head is to be regarded as a cell nucleus, so that Miescher gained possession of material in great quantity which was admirably adapted to a chemical examination of the

cell nucleus. He found the spermatozoa heads free from protein and made up almost entirely of a single chemical [illegible] Khorus. This substance was the first known member of a [illegible] group of acids now called Nucleic Acids, some member of which is present in the nucleus of every animal and plant cell. While the biological distribution of the nucleic acids is very wide nearly all the chemical information which we possess of the members of this group was originally acquired from researches executed with two substances, one of which is prepared from yeast (Yeast Nucleic Acid) and the other from the Thymus gland (Thymus Nucleic Acid). Both of these substances were submitted to chemical examination for the first time by Kossel, whose widely known and highly esteemed investigations culminated in the [illegible] groups so that when it is submitted to hydrolysis with mineral acids it produces the six substances, guanine, adenine, cytosine, thymine, phosphoric acid and sugar.[2]

[illegible] A. Kossel und A. Neumann 1894). Darstellung und Spaltungsprodukte der Nucleinsäure. Ber. d. d. chem. Ges. 27, 2215.

Kossel also showed that yeast nucleic acid contains

respect is made in the following table.

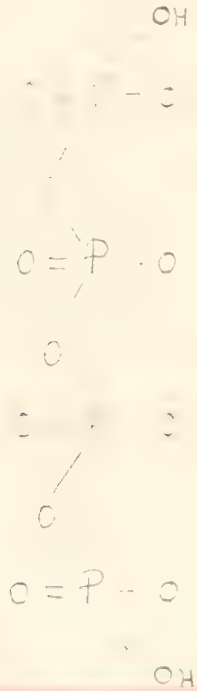
Hydrolytic Products	
Thymus nucleic acid (animal nucleic acid)	Yeast nucleic acid (plant nucleic acid)
Adenine	Adenine

The facts embraced in this table are among the most firmly

stitute the source from which all the more recent discussions of

(3) A. Ascoli (1900). Ueber ein Nuclein spaltendes Ferment der Hefe. Nuclease. Zeits. f. physiol. Chem., 31, 161.

the subject have proceeded. They serve to characterize the [illegible] substances of this class have been found to yield one or the other of these sets of hydrolytic products. To resolve the constitution of nucleic acids it therefore remained only to find the order in which the six groups are arranged. In this direction also Kossel made the first advance[5], when he obtained polyphosphoric acid from yeast nucleic acid and thus furnished a strong indication that the nucleic acid has at the base of its structure some such complex as that [illegible]



⑤ A. Kossel; (1900) Zur Kenntn. Nucleinsäure. Zeitschr. f. physiol. Chem., 31. ~~161~~ 428

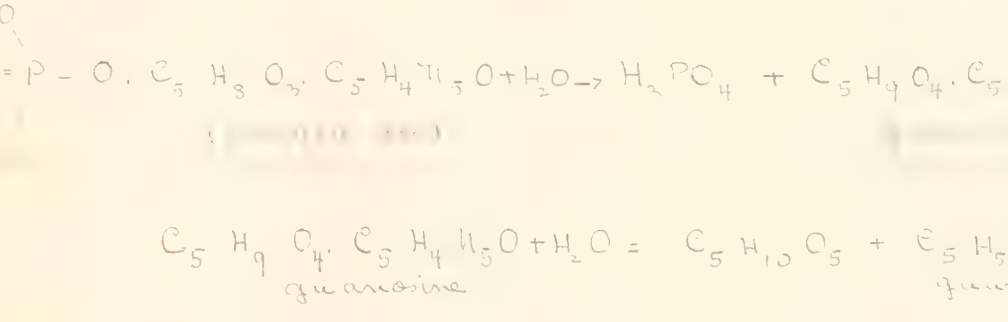
At this point the subject assumed a little complication [illegible] guanylic acid, which is obtained from the pancreas and which is believed to yield on hydrolysis phosphoric acid, guanine, pentose and glycerine. This conception, to which the discovery of guanylic acid gave rise was considerably simplified when Steudel[1] found that the substance does not produce glycerine on hydrolysis. But [illegible] scarcely be physiologically related to the nucleic acid of Animal cell nuclei since it contains a pentose group, that is to say, the carbohydrate group of plant nucleic [illegible]

[illegible] it was found that guanylic acid is not confined to the pancreas, but is very widely distributed in animal

(6) J bar Barq (1898). Die Guanylsäure der Pankreasdrüse und ihre Spaltungsprodukte. Zeits. f. physiol. Chem., 26, 133.

1. Steudel (1907) Ueber Guanylsäure aus der Pankreasdrüse. Ibid 53, p 534.

glands,[5] and shortly afterwards Levene and Jacobs[6] solved its chemical constitution. By submitting the substance to neutral hydrolysis under pressure they found that it [illegible] nucleoside called guanosine, which in turn yields pentose and guanine.



$$HO-P(=O)-O.C_5H_8O_3.C_5H_4N_5O + H_2O \rightarrow H_2PO_4 + C_5H_9O_4.C_5H_4N_5O$$

$$C_5H_9O_4.C_5H_4N_5O + H_2O = C_5H_{10}O_5 + C_5H_5N_5O$$

(guanosine; guanine)

The guanylic acid is representative of a class of substances called <u>nucleotides</u>, i.e., compounds in which groups of phosphoric acid and nitrogenous rings are linked by carbohydrate groups.

Levene and Jacobs[7] then applied to yeast nucleic acid the method which they had successfully employed with guanylic acid, and found that by neutral hydrolysis under

[illegible] Salkowski (1900) Einige Untersuchungen über ein Nucleoprotein der Tuberkelbacillen [illegible]

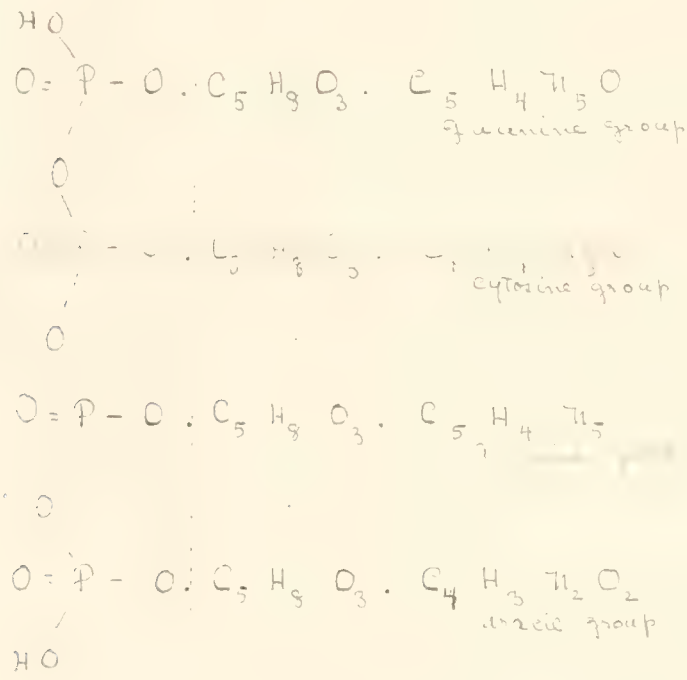
[illegible] Levene and [illegible] Jacobs (1909). Zur Chemie der Pankreasnucleoproteide. Biochem. Zeits., 10, 221

[illegible] P.A. Levene and W.A. Jacobs. 1909, Ueber Guanylsäure. Ber. d. deutsch. chem. Ges. 42, 2469

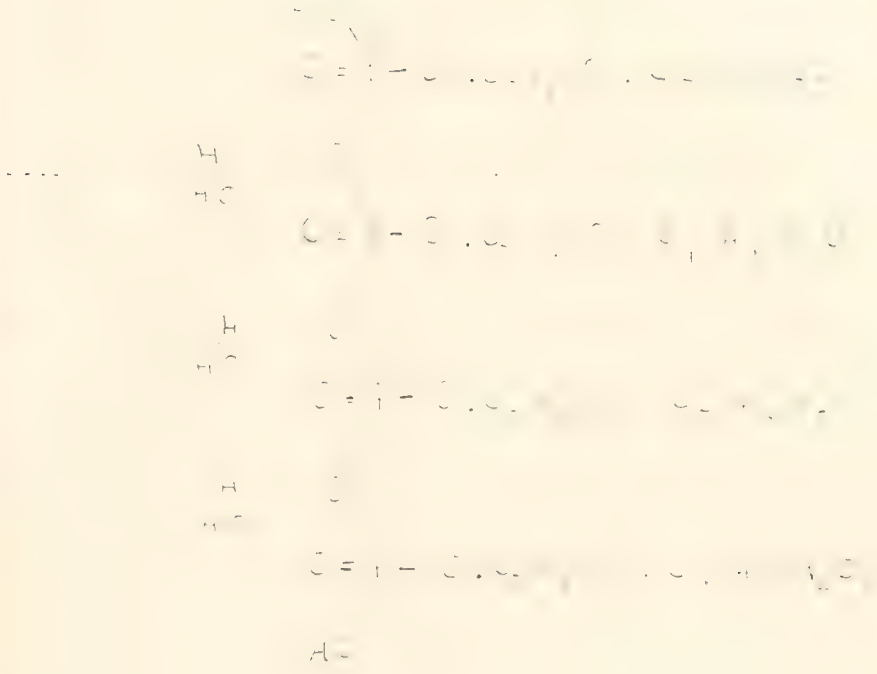
[illegible] Levene and W.A. Jacobs. (1909) Ueber die Hefe-Nucleinsäure. Ibid 42, 2474

[illegible] Levene and W.A. Jacobs (1909) Ueber die Purine in der Thymusnucleinsäure. Ibid

pressure the substance loses phosphoric acid and yields four nucleosides; one of which is identical with the nucleoside guanosine, similarly obtained from guanylic acid. [illegible] is to yeast nucleic acid as guanylic acid is to [illegible]. Yeast nucleic acid is a tetra-nucleotide and is composed of four mono-nucleotide groups and has in the following formula:



The dotted line of the formula above indicates how four nucleotides must of necessity be produced when nucleic acid is decomposed in such a way as to completely lose its [illegible] indirectly. But if hydrolysis should occur in such a way as to bring about a disruption of the phosphoric acid groups [illegible] other way, four mono-nucleotides would be formed, one of which must be identical with guanylic acid. This is seen in the following diagram:-

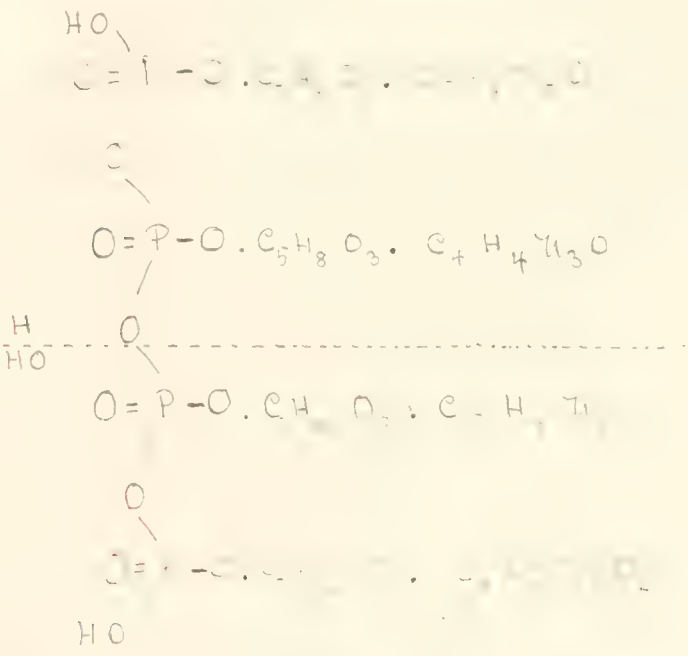


To obtain guanylic acid from yeast nucleic acid would therefore furnish a strong and independent confirmation [illegible] have assigned to yeast nucleic acid, and we even venture to suggest that the conformation would occur at the point of the formula where it is most needed.

The formation of guanylic acid from yeast nucleic acid by the action of the ferments contained in a pre-

by Jones[1], but the results which he described must be regarded as more or less accidental, or rather the experiment ... which it is difficult to control and can be repeated with only occasional success. This will be clear from the following considerations.

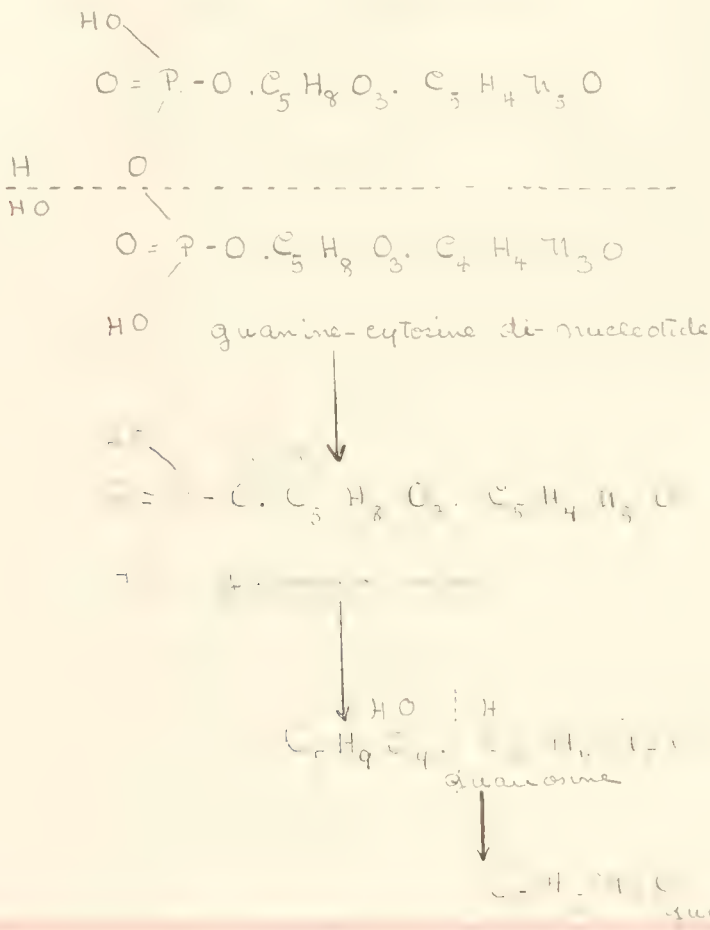
When yeast nucleic acid is submitted to the action of an extract of fresh pig's pancreas it is first decomposed into two di-nucleotides.



[1] W. Jones (1912) On the formation of guanylic acid from yeast nucleic acid J. Biol. Chem. 12, 31.

This phase of the decomposition can now be controlled and possession of the two products can be conveniently gained. One of these has been obtained in very pure condition and yields by acid hydrolysis both guanine and cytosine, but neither adenine nor uracil; while the other di-nucleotide yields similarly both adenine and uracil, but neither guanine nor cytosine. Each substance, therefore, contains both a purine and a pyrimidine group, and showed [illegible] mild hydrolytic agencies. Experiment has shown this to be the [illegible] (nuclease) And they represent the first phase of the enzymatic hydrolysis of yeast nucleic acid. By continued action of the extract of pig's pancreas other ferments evidently of less activity split the di-nucleotides. Are further decomposed into their constituent mono-nucleotides, one of which is guanylic acid. This constitutes

[illegible] nucleotides in consequence of which the corresponding nucleosides are formed. This is the third phase of the enzymatic hydrolysis of yeast nucleic acid. This progressive decomposition of yeast nucleic acid through di-nucleotides, [illegible] of purine bases is shown in the following diagram.



But it happens that when the extract of pigs pancreas is allowed to digest at 40° before adding the yeast nucleic acid its ferments are gradually destroyed and in an order inverse to that in which they are exerted upon nucleic acid; so that by properly selecting the duration of this preliminary digestion one should be able to gain possession of a ferment [illegible] given point but no farther. In practice, however, this is exceedingly difficult (especially as the duration of digestion is not the only factor which determines the destruction of the ferments,) for one will either allow the fresh pancreas to digest for too short a time, in which case its subsequent action on yeast nucleic acid will proceed beyond the de- [illegible] pancreas extract to digest for too long a time and its subsequent action on yeast nucleic acid will not proceed as far as the formation of guanylic acid.

In the great majority of experiments, therefore guanylic acid will either not be found present among the pro-

ducts of digestion, or will be contaminated with the guanine-cytosine di-nucleotide, since the method of isolation is the same for both substances.

[illegible] hydrolysis of yeast nucleic acid and its decomposition products [illegible] was clearly shown in a study of the action of rabbits liver extract on guanine. A fresh aqueous extract of this tissue [illegible] completely, so that after digesting with an extract of this tissue for a short time the guanine will have completely disappeared and in its place will be found an equivalent [illegible].

[illegible] This reaction shows that the ferment "guanase" is present in yeast extract [illegible] we allowed to digest at the body temperature [illegible] added to such a digested extract will remain un-

altered and can be recovered at the end of the digestion. [illegible] cannot be found.

Not only are these so called "nuclein" ferments destroyed when their solutions are allowed to remain [illegible] sensitive to the "poisonous" effect of the substances [illegible]. These two matters might lead one to suspect that definite results in this field are difficult or even impossible to obtain. [illegible] the possible absence of "poison" can obtain results as precise and uniform as are to be secured in any [illegible] in fact one of the most desirable consequences of the work which is here described was secured by taking advantage of the poisonous action exerted by yeast nucleic acid upon the ferments which decompose it. As already stated a fresh aqueous

among the products and completely identified.

one of the intermediate products of the enzymatic

either to bring about conditions under which the

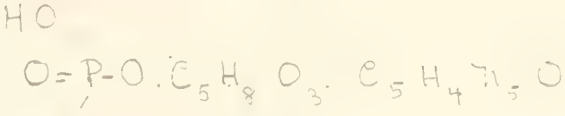
product in question is formed as an end product

or to find in nature a source of enzyme activity

composition product of yeast nucleic acid and one

of the substance is guanylic acid two methods

from the products is effected by a process so simple as scarcely to admit of failure. The guanylic acid thus obtained has been thoroughly identified with the guanylic acid originally found by Bang in the pancreas [12] and yeast-nucleic acid by Levene and Jacobs, and has been found to possess the composition required for the formula,



[12] Ivar Bang: loc. cit.

liberates its entire phosphoric acid by mild [illegible] in alcohol, responds to the pentose color reaction of Tollens[13], and produces the beautifully crystalline brucine salt which Levene and Jacobs[14] obtained with the guanylic acid of the pancreas. In short there is no observable difference between the substance which we obtained by the enzymatic decomposition of yeast nucleic acid and the pancreatic guanylic acid, whose structure Levene and Jacobs have [illegible]

[illegible]

[13] B. Tollens (1896) Ueber den Nachweis der Pentosen mittels der Phloroglucin-Salzsäure-Reaktion. Berichte d. chem. Ges., 29, 120.

[14] P. A. Levene and W. A. Jacobs (1912). On guanylic acid. J. Biol. Chem., 47, 151.

A mixture of equal weights of water with carefully trimmed and finely ground pigs pancreas was placed in a closed vessel with enough chloroform [illegible] tissue might become thoroughly penetrated by the chloroform. Unless this precaution be taken, the products are almost sure to putrefy when brought to the body temperature. The material was then allowed to digest in a thermostat at 40° for two weeks. [illegible] precipitated and all ferments present are de-

stroyed except the one that yeast diesterase nucleic acid
i.e., forms the di-nucleotides. The fluid was cooled and
filtered when a perfectly clear pale yellow solution
was obtained which [illegible]
with yeast nucleic acid (1.5 g. of the nucleic acid for
each 100 cc. of extract) and the product allowed to re-
main at 40° for a convenient length of time more
[illegible]
the nucleic acid could be shown by the failure of sul-
phuric acid to produce a precipitate with a portion of
[illegible]
exert the slightest activity on thymus nucleic acid
[illegible]
fermentation.

The two di-nucleotides can be separated from var-
ious other constituents of the digestion products and from
one another by the following method. The fluid is

heated to boiling, filtered from a small amount of coagulum and kept near the boiling point as it is treated with a solution of neutral lead acetate as long as the reagent forms a precipitate in the hot fluid; but care should be taken that no considerable ex- [illegible] nucleotides will be precipitated. It is not at all difficult to make this precipitation accurately, because there is a wide margin between the point where the precipitation of phosphates, nucleic acid, coloring matter etc. ends and the point where the precipitation of the di-nucleotides begins. A number of ob- [illegible] a very pale fluid that can easily be filtered from the bulky lead precipitate by means of a Büchner filter, and which forms a heavy granular lead precipitate when treated at boiling point with an excess of neutral lead acetate. This lead precipitate which settles rapidly increases in amount as the solution

[illegible] can be separated by decantation. It consists of a mixture of the lead salts of the two di-nucleotides. By suspending these lead salts in hot water and de-

[illegible] free di-nucleotides is obtained. The only known method of separating these di-nucleotides from one another is [illegible] salts when treated with alcohol; the one substance [illegible] from the other completely precipitated. [illegible] the aqueous solution of di-nucleotides was filtered from lead sulphide, concentrated at 50° under diminished pressure, and treated with a small quantity of an aqueous solution of potassium acetate. The potassium salts are slowly formed and the solution becomes gelatinous as may have been expected but before gelatinization has proceeded far the products are poured [illegible]

the one dinucleotide is precipitated and settles rapidly while the other dinucleotide remains suspended for hours, but on standing over night it becomes converted to a resin leaving a perfectly clear liquid. This resin which entrapps all the coloring matter of the fluid may be hardened by successive treatments with air-

[illegible] fluid may [illegible]. The writer recently repeated the above process to effect a sharp separation of the two substances and should not be surprised to find that the properties of each are slightly [illegible] the other. Nevertheless the separation is sufficiently [illegible] of the compounds viz. that each yields only one [illegible]

[illegible]

The substance precipitated upon [illegible]

acid

potassium acetate and alcohol and dried over sulphuric

is easily soluble in water and laevorotatory to bo-

tatory.) The substance was dried to constant weight

at 105° and analyzed for potassium, phosphorus and

nitrogen, by the methods of Neuman[15] and Kjeldahl

	I	II.	III	$C_{19}H_{24}N_8P_2O_{15}K_2$
Potassium	9.9	10.2	10.4	10.8
Phosphorus.	7.75	7.70	7.79	8.33

Several weighed portions of the di-nucleotide, one gram

each, were heated with 10 cc of 5%

densing tube and immersed in boiling water

[15] A. Neuman. (1902). [illegible] Ztschr. f. physiol. Chem., 37, 115.

the product was heated with an excess of ammonia until hot, and after cooling the coarsely granular guanine was filtered off, dried and weighed. The ammoniacal filtrate from guanine was then treated with magnesia mixture for the precipitation of phosphorus and [illegible] nitrate. In case a small precipitate was formed it was decomposed and tested for adenine in the usual way. The specimens of ammonium magnesium phosphate were dissolved in nitric acid and precipitated in turn as ammonium [illegible] phosphate. The latter was weighed and the phosphorus calculated. It will be seen that the small quantity of phosphorus liberated by mild hydrolysis is approximately half the total phosphorus of the compound, showing that one is here dealing with a mixed nucleotide containing both a purine and a pyrimidine base. Each specimen of guanine was converted into the chloride which was

to consist of the beautifully feathery needles characteristic of guanine chloride. Some of the specimens were analyzed.

	Theoretical Guanine	Found Guanine	Theoretical Guanine for Yeast Nucleic Acid	Found Adenine	Theoretical for Total Purines	Theoretical for Herring Preparation	Found Purines
I	20.29	21.70		0	8.33	7.17	3.20
II		20.92		0			3.35
III		21.26		Trace			3.26
IV		22.00		0			3.60
V		21.07		Trace			4.04

Twenty grams of the di-nucleotide were heated for three hours in an autoclave at 130° with 2 5% sulphuric Acid and the products examined for hypoxanthine bases by the well known silver method. A characteristic specimen of

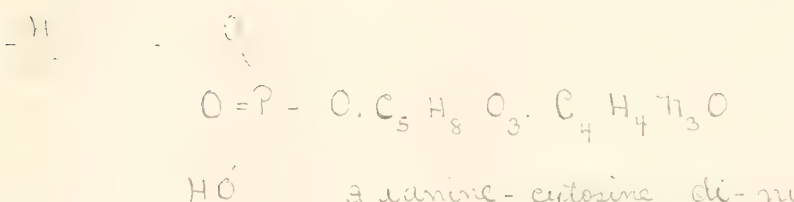
Theoretical for

$C_4H_5N_3O.C_6H_3N_3O_7$

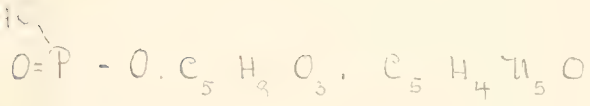
N 29.11

of its phosphorus upon mild acid hydrolysis, and yields nearly twice as much uracil as can be obtained from yeast nucleic acid, but at most only a trace of

The experiments described in the preceding chapter show that the first decomposition which yeast nucleic acid undergoes results in the formation of a di-nucleotide which contains the whole of the phosphoric acid of the yeast nucleic acid and is an expression of a weak point in its molecule and that the di-nucleotide will be the first product which undergoes enzymatic hydrolysis under the influence of any appropriate [illegible]. Its enzymatic hydrolysis moreover suggests very clearly that the second stage in the [illegible] of yeast nucleic acid will consist in a similar hydrolysis of the di-nucleo



guanine-cytosine di-nucleotide



discontinuing the digestion or by "poisoning" the ferments with an excess of initial yeast nucleic acid. This result may also be reached by the employment of a pancreas extract that has been submitted to a short preliminary digestion at 40°. But in the execution of all [illegible] particular analytical methods must be applied for the isolation of the product. It happens however that [illegible] capable of forming guanylic acid from yeast nucleic acid, and while the decomposition proceeds soon to the [illegible] greater amount than either of its two decomposition products, so that its analytical separation can offer no difficulty. The following method is easily executed and has been found to yield uniform results.

A mixture of 200 grams of Liebig's commercial yeast powder, 2000 cc. of water, and 60 grams of

commercial yeast nucleic acid were allowed to digest at 40° in a closed vessel, with enough chloroform to prevent putrefaction. (In addition, a control was made with the same materials except that no sulphuric acid was added). From time to time small portions of the fluid were withdrawn and used for nucleic acid estimation with sulphuric acid. The amount of nucleic acid precipitated in this way was found to diminish continually as the digestion proceeded until the fifth day, when the failure of sulphuric acid to produce any precipitate at all with the product, showed that the nucleic acid had entirely disappeared. The clear fluid was still brown, was neutralised as carefully as possible to the sediment of yeast detritus and allowed to remain in an ice chest over night, when there occurred a copious deposition of flocculent material. On the contrary the fluid from the control gave no precipitate at all when cooled, showing that the

substance in question originates from the nucleic acid employed and not from the yeast powder. Moreover the gradually increasing amount of this insoluble substance which is produced as the digestion proceeds in connection with a corresponding gradual disappearance of the initial nucleic acid indicates that we are dealing with a physiological agent of whose activity time is a constant function.

In order to isolate guanylic acid from the digestion products it is only necessary to filter off and manipulate the substance formed by cooling the material, but the yield can be greatly increased by the addition to the fluid before cooling one tenth of its volume of absolute alcohol. Under these conditions the insoluble substance is not only precipitated in greater amount but can be [illegible] more easily and after drying in the air has a tendency to [illegible]

the substance may be obtained by concentrating the filtrate to one half its volume at 50° under diminished pressure and again cooling in the ice chest after the addition of one tenth of its volume of absolute alcohol.

The material obtained by the above method is very difficultly soluble in cold water and may be freed from coloring matter and adherent soluble impurities by deposition from a hot aqueous solution as it cools.

[illegible]

about 80 parts of boiling water and 250 parts of water at the room temperature. It is a mixture containing a large proportion of guanylic acid with much [illegible]

Small quantities of both calcium and magnesium are also present in the material, which though maintaining a fairly constant composition as it is successively "crystallized" does not contain its constituents in a proportion that accords with any assign-

I. .2065 g. required 10.50 cc of standard sulphuric acid
(1 cc = .0037 g. Mg)

II. .2078 g. required 10.55 cc of standard sulphuric acid

III .1367 g " 6.81 cc " " " "

IV. 1861 g " 9.39 cc " " " "

V. .6595 g. gave .2660 Mg $NH_4PO_4 \cdot 6H_2O$

VI. .6530 g. " .2621 "

VII. 5081 g. " .2080

VIII. .4823 g. " 1990 "

IX. .698 – gave .1200 g. Mg $NH_4PO_4 \cdot 6H_2O$ (for magnesium)

X .[illegible]

	Found			
	I	II		
H	18.83	18.78	18.43,	18.66
P			5.24	5.4 5.39

.7563 grams of substance were heated for an hour at 100° with 10 cc of 8 % sulphuric acid, and [illegible] The guanine was precipitated with [illegible] dried and weighed. Guanine - .2570

The filtrate from guanine was treated with a solution of silver nitrate in ammonia, and the very small precipitate was decomposed and carefully examined for adenine but with negative results. This complex mixture contains therefore only the purine group, and it is possible to obtain from it only guanine derivatives. By using

the following method the substance may be separated into these said derivatives viz. guanylic acid, guanosine and guanine.

A solution of the material in warm water is made faintly alkaline with sodium hydroxide and the [illegible] is acidified with acetic acid and treated with neutral lead acetate. The guanylic acid is thrown down as [illegible], granular and pure, which after filtration* and thorough washing with boiling water is suspended in cold water and decomposed by the passage of sulphuretted hydrogen. The lead sulphide is filtered off, the excess of sulphuretted hydrogen is removed from the cold solution with an air current and the fluid is concentrated at 50° under diminished pressure. Upon cooling to zero a white granular [illegible] free guanylic acid, but occasionally contains a small of guanine. The latter may be removed by

* The filtrate is used for the preparation of guanosine, see below.

treating the product with warm water, filtering and treating the cold aqueous solution with a large excess of absolute alcohol. Pure guanylic acid is precipitated in [illegible] white flocculent form, which is washed with alcohol and dried in a dessicator over sulphuric acid.

[illegible]

is for this reason a little low and its nitrogen correspondingly high.

[illegible] to a constant weight at 70° gave the following [illegible]:

I .5314 g. gave .3272 g. of $MgNH_4PO_4 \cdot 6H_2O$

II .4050 g. gave .2491 g. of $MgNH_4PO_4 \cdot 6H_2O$

II. .1471 g. required 7.98 cc of standard acid (1 cc = 0.003695 gram H.)

III. .1504 g " 8.24 cc "

	Theoretical for $H_2PO_4 \cdot C_5H_8O_5 \cdot C_5H_4N_3O$	Found I	Found II
H	4.61		
P	9.37		

Various [illegible] gave upon ignition and [illegible] to constant weight [illegible] the following analytical results.

I. [illegible] g required 14.58 cc. of standard acid (1 cc = 0.003695 H)

II. [illegible] g " 8.24 cc "

III. [illegible] g " 10.88 cc "

IV. [illegible] g gave .3431 g. $Mg_2H_4P_2O_7 \cdot 6H_2O$

V. [illegible] g " .3571 g. "

VI. [illegible] g .3412 g "

VII. [illegible] g .3006 g

	Theoretical for	Found				
N	19.25	19.35	18.86	18.98		
P	5.55			5.09	5.37	5.21	5.21

.3500 gram of substance was heated for an hour with [illegible] The product was made alkaline with ammonia and the precipitated guanine filtered off. It weighed 0.1499 gram.

	Theoretical for	Found
Guanine	71.43	37.47

A drop of the filtrate from guanine failed to give a precipitate with silver nitrate in ammonia. Hence

adenine was not present. The rest of the fluid was then treated with an excess of magnesia mixture and the precipitated magnesium ammonium phosphate was filtered off and weighed.

.3800 gram gave .2370 gram of $MgNH_4PO_4 6H_2O$.

	Theoretical for Guanylic acid	Found
[illegible]	[illegible]	[illegible]

[illegible] appeared above next [illegible] of the [illegible] acid of the compound [illegible] Therefore the substance does not contain [illegible] guanidine group

A portion of guanylic acid dissolved in water was treated with a small amount of a solution of potassium acetate. And the acid potassium salt of guanylic acid was precipitated by the addition of alcohol. The Total phosphorus of the substance was

molybdic solution, and then with mag-
nesia

The original filtrate from lead guanylate contains guanosine, which can be easily isolated in the following way. The solution is treated with sulphuretted hydrogen, filtered from lead sulphide and evaporated [illegible] neutral lead acetate to this concentrated fluid usually precipitates a small amount of guanylic acid which remained in solution after the former treatment with lead acetate, owing to the greater volume of fluid, [illegible] a finely crystalline specimen of guanosine is to be obtained. [illegible] alternately with small portions of lead acetate and ammonia as long as either reagent produces a precipitate and the precipitated lead compound of guanosine is suspended in hot

water and decomposed with sulphuretted hydrogen. After evaporating the filtrate from lead sulphide [illegible] concentrated fluid in the ice closet, guanazine is deposited [illegible] upon recrystallization from hot water form individual long thin plates with sharp edges.

I. .2176 gram required 12.82cc of standard sulphuric acid (1cc = .0 03695 gram N).

II. [illegible] gram of the substance [illegible] .0472 gram [illegible] .

	Theoretical for $C_5H_9C_4 \cdot C_5H_4N_5O.2H_2O$	Found I	Found II
N	21.94	21.76	
C	11.3		10.7

The formation of both guanosine and guanylic acid from yeast nucleic acid of course does not [illegible] but guanylic acid alone is first formed and that part of this is decomposed forming guanosine. The two substances then combine to form the compound which has been described, whose physical [illegible] the other products of digestion, thus furnishing a [illegible] and guanylic acid.

Conclusion

The principle contention of this dissertation is that yeast nucleic acid contains a guanylic acid identical in all respects with the guanylic acid found in the pancreas [illegible] specimens of the substances side by side, when their properties, even down to unessential details were found to coincide throughout. While the identity of the two guanylic acids distributions are almost impossible to explain, the guanylic acid of the pancreas contains and is characterized by a ketose group ($C_5H_{10}O_5$), whereas the nucleic acid not only of the pancreas, but of all other animal tissue is equally characterized by its

are scarcely possible that the guanylic acid of the pancreas is in any way derived from a nuclein precursor in this gland. The easy formation of guanylic acid from yeast nucleic acid and a substance constantly present in animal food, seriously suggests that the guanylic acid of the pancreas is formed from the food-stuffs and not from the tissues of the body. This conclusion, however, is based upon a purely speculative basis, as we remember that the production of guanylic acid from yeast nucleic acid has also been an experimental basis, and that the amount of guanylic acid obtainable from an animal pancreas is in direct proportion to the amount of plant food which it contains. This point has been illustrated in the cases of the pig and the ox, the former having a diet rich in vegetable food, whereas the latter is fed almost entirely upon meat.

latter. Moreover, in support of the hypothesis here proposed it may be stated that the guanylic acid of the pancreas which has been frequently described in the literature is indeed not guanylic acid at all but a compound or mixture of guanylic acid with [illegible] yielding compound obtained by the action of yeast on yeast nucleic acid, which has been described in this paper.

Biography.

The author of this dissertation was born in Radnor, Pennsylvania in 1885. She received the degree of A.B. from Bryn Mawr College in 1907 and studied chemistry there for two years as a post graduate. She taught Chemistry and Physiology in the Philadelphia High School for Girls during the year 1910-1911, and was assistant in Chemistry at Barnard College the following year. The year 1912-1913 she spent as a graduate student in Chemistry at the Johns Hopkins University, where she received a scholarship in Chemistry. She has studied during the present year in the Laboratory of Physiological Chemistry of the Johns Hopkins Medical School.

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